BEHAVIOR OF THE CELL SURFACE DURING CLEAVAGE. VII. ON THE DIVISION MECHANISM OF CELLS WITH EXCENTRIC NUCLEI

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The conventional diagram of a typical cell shows the nucleus in a central location, but actually in many cases the nucleus does not occupy the exact geometrical center of the cell. The present paper deals with the mode of division of cells with excentric nuclei, especially in connection with the general theory of the mechanism of cell division proposed in a previous paper of this series (Dan, K., 1943b).

REVIEW OF THE PROPOSED THEORY

The salient features of the theory are as follows: (1) The asters of sea-urchin eggs are spiny spheres with numerous gel rays radiating out in all directions. (2) Cleavage is brought about as a result of the pushing apart of two such radiate spheres by the autonomous elongation of the spindle (Fig. 1). Applying this concept, a cell in the resting stage can be regarded as a mass of fluid cytoplasm contained in a gelated cortex. When two radiate gel spheres which have developed and reached their maximal size within this fluid cytoplasm are pushed apart by the spindle, the original fluid mass will be divided into two portions which are surrounded externally by the cortex and supported internally by the spiny spheres (Fig. 1). Observations on adhering kaolin particles have shown that the surface of the furrow expands during the latter half of the division process (Dan, Yanagita and Sugiyama, 1937; Dan, Dan and Yanagita, 1938). We can account for this expansion phase if we visualize a definite amount of fluid cytoplasm being divided by the above mechanism. As the asters are pushed apart, the cortical layer covering the cleavage furrow will be sucked in between them and will show a linear stretching along the sides of the furrow. But actual observations have also shown that, at the beginning of the cleavage process, before the expansion phase, the furrow surface undergoes a phase of shrinkage. This initial shrinkage phase may be explained by two more details of the division process. (3) The astral rays cross at the future cleavage plane (Fig. 1, Stage 2), and (4) the rays are anchored to the cortex of the cell. As was fully discussed in the previous paper (Dan, K., 1943b), the rays exert a traction effect upon the cortex of the egg and this effect causes the initial shrinkage, whereas the later expansion of the furrow surface is due to the suction effect mentioned above. Let us consider this more fully. If the underlying spindle elongates while the tips of the individual rays are attaching to the cortex, the loci along which the tips of the crossing rays will move are practically the same as the loci of the apices of triangles made by the crossing rays, the spindle (i.e., the base of the triangle) and a line connecting the tip of each ray with the astral center of the opposite blastomere. Such loci form a curve directed toward the base line and gradually approach-
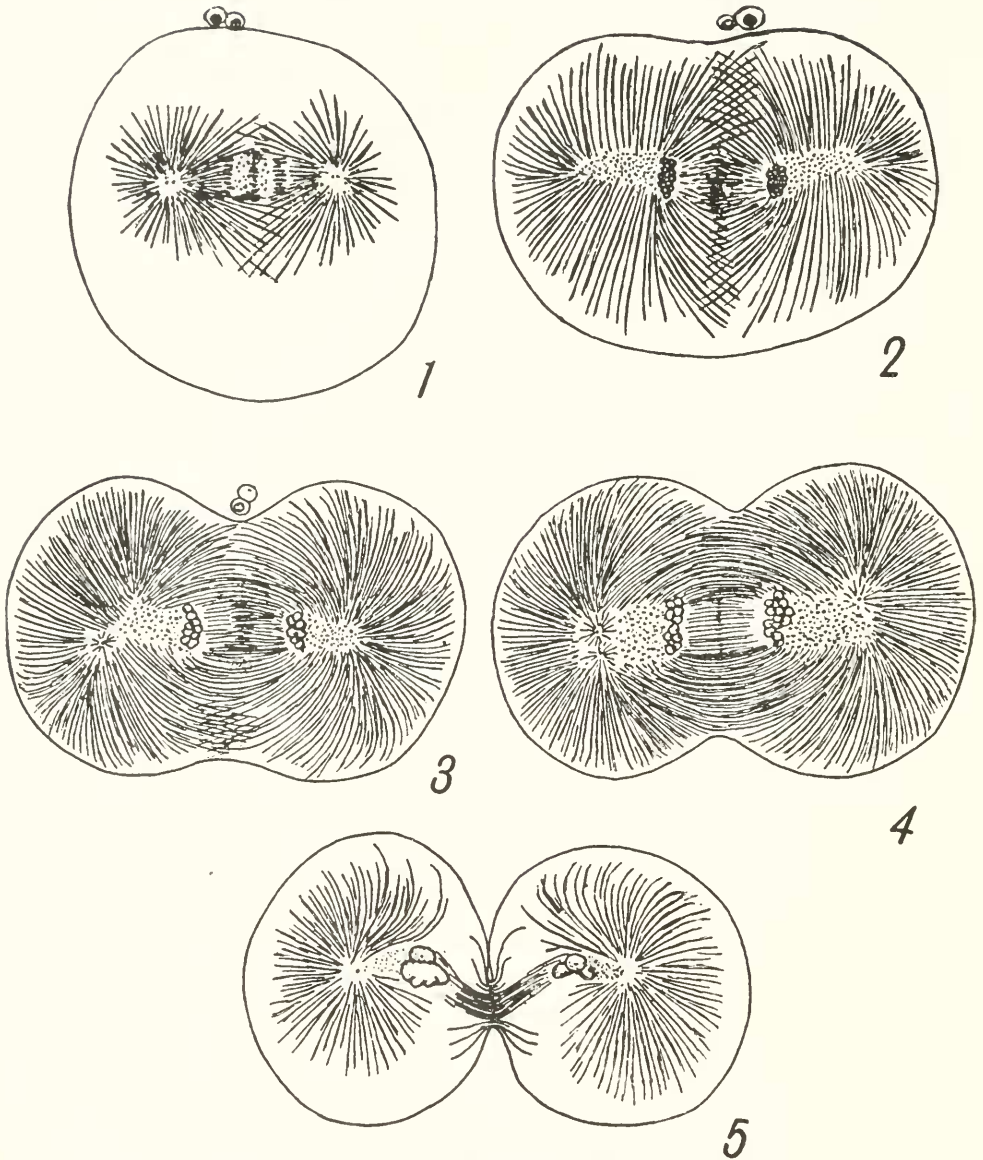
FIGURE 1. Division of the eggs of *Cerebratulus lacteus*. Reproduction of Yatsu's Text Figure C. Legends from Yatsu. "Five stages of the first cleavage, × 400. 1. Anaphase of the first cleavage mitosis. Crossing of rays, and the position of the spindle. Difference in distance between two centrioles at either end. 2. Telophase of the first cleavage mitosis. The centrosome has enlarged and been bent downwards. The crossing of rays has begun to loosen. A faint indication of fountain figure is seen at the pole regions. 3. Telophase; a vertical section through the poles and a centriole. Constriction has begun on both the animal and vegetative sides. Sheath spindle has been formed. Fountain figures at the poles of the spindle have become more distinct. 4. Telophase, a horizontal section through three centrioles (about the same stage as 3). Sheath spindle is very well formed. Centrosome has greatly enlarged. The distance between the rows of karyomeres is approximately the same as the original length of the spindle. 5. Late telophase (a vertical section). Sheath rays have been formed. Fountain figure in the equatorial rays."

ing a vertical line bisecting the base (Fig. 2). As the triangles become less equilateral the curve becomes greater. On the contrary, if two astral rays meet exactly on the median plane an equilateral triangle is obtained and the locus of the apex coincides with the vertical line bisecting the base. Seven such loci are shown in Figure 2. As an example, if a pair of symmetrically crossing rays is considered, two unequilateral triangles which are mutually mirror images are obtained and their apices will converge toward the mid-line as the spindle elongates. This means that the surface included between the apices will be made to shrink. The situation is
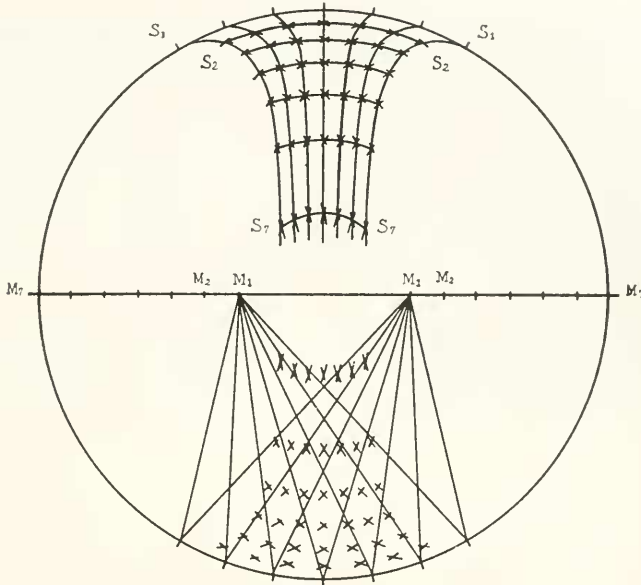

FIGURE 2. Loci of seven pairs of rays which meet end to end and which are attached to the cortex when the spindle elongates. The lower half of the figure shows the positions of the tips of the rays for seven different lengths of the spindle such as are indicated on the horizontal line. The upper half shows the same groups of points in terms of the surface. Notice that the rate of shrinkage of the surface is fast in the beginning and slow later, while the rate of the inward shift of the surface is slow at the beginning and fast toward the end. Note also that the wider the range of crossing of the rays, the greater is the rate of shrinkage. The innermost position of the surface (S_7S_7) indicates that a complete division of the cell cannot be achieved by this mechanism alone.

most clearly demonstrated by an experimental model, shown in Figure 3. Since both the traction effect of the astral rays and the suction effect in the cortical layer of the furrow are equally direct results of spindle elongation, there is no contradiction of the statement made under (2).

Finally it must be considered how and when the traction effect is relayed over to the suction effect. To explain this another feature of the division figure must be examined. (5) A fountain-figured bending of the polar rays takes place (Fig. 1, Stages 3 and 4). If the rays were rigid and not pliable, as soon as the spindle elongated the suction effect would be felt at the cleavage plane and there would be

no time for the traction effect to play its role.   But actually the rays are pliable.
Therefore when the spindle elongates and pushes the asters against the polar sur-
faces, the rays yield to the pressure and bend in a fountain figure.   By this bending
of the rays, the spindle elongation is not transmitted directly to the cell contour but
is buffered.   Once the bending of the polar rays reaches the maximal degree, how-
ever, further lengthening of the spindle brings about the suction effect.   Thus, even
though the dynamic cause of cell division is single—namely, the spindle elongation—
its effect on the cell surface involves two factors which come into play in succes-
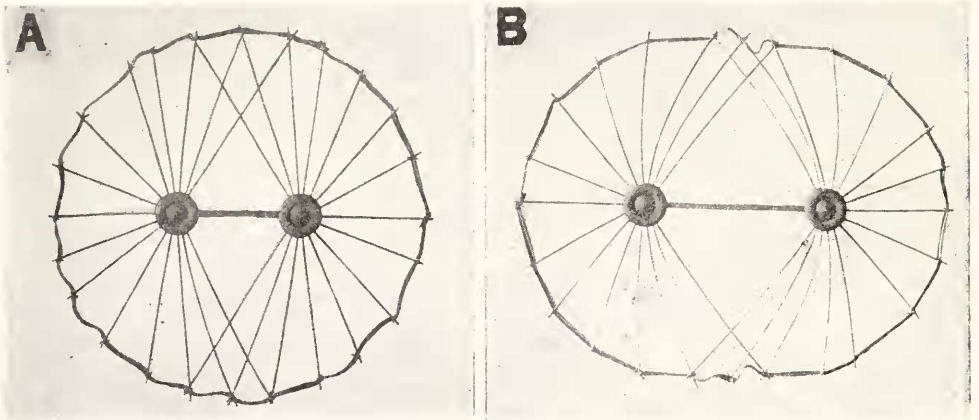

FIGURE 3.   A model imitating the initial shrinkage phase of a cell with a central spindle.
Astral centers are represented by wooden wheels in which bamboo rods are firmly planted imi-
tating astral rays, which, in turn, are tied to a rubber band simulating the cell membrane.   A
wooden bar which represents the spindle is also firmly fixed to each wheel, the bars from the
two wheels overlapping in the spherical condition (A).   The bars have small holes along their
lengths so that they can be fixed in any desired length by putting a pin through these holes
(B).   In B, note (1) the flattening of the furrow regions, (2) the shrinkage of the furrow sur-
face, (3) fountain-figured bending of the polar rays and (4) consequent stretching of the polar
surface.   Note also that (5) the spindle is straight after the elongation.

sion.   It may be pointed out in passing that the fountain-figured bending of the polar
rays theoretically ought to bring about an expansion of the overlying surface (polar
surface).   As a matter of fact, the polar surface does expand from the beginning of
the cleavage process, in contradistinction to the furrow surface.

THEORETICAL CONSIDERATION OF THE DIVISION MECHANISM OF CELLS WITH EXCENTRIC NUCLEI

Returning to the division of cells with excentric nuclei, one might say that there
are only two known facts which are widely accepted today.   One is that the mitotic
figures resulting from excentric nuclei are also situated excentrically in the cells.
The other is that the cleavage furrow appears first at the side of the cell nearest to
the excentric mitotic figure, later spreads around the two sides and finally com-
pletes its circuit at the opposite side of the cell from that at which it was first formed
(Ziegler, 1898).

In order to repeat the observations, the authors used the eggs of the sand-dollar, *Astriclypeus manni.* In these eggs the mitotic figures are excentric toward the animal pole, and the furrow starts to appear from there. The astral rays are much better defined than in other forms. They are clearly seen crossing all along the median plane despite the fact that the rays going to the animal hemisphere are shorter than those distributed in the vegetal hemisphere. When the cell bodies begin to elongate for cleavage, the polar fountain figures of the rays are also seen quite definitely. Let us see if these division features of an excentric egg can be harmonized with the concepts of the proposed theory.

In the first place it might be anticipated that the furrow would appear earlier at the side nearer the mitotic figure (the animal pole in *Astriclypeus*), because, during the initial stage of cleavage when the rays are crossing, for a given elongation of the spindle the tips of the shorter rays would be pulled in more than those of the longer rays. Later, after the crossing of the rays is dissociated, a wider gap would form between the parts of the asters with shorter rays than between those with longer rays, resulting in a deeper furrow in the region of the shorter rays. So far the concepts are quite adequate, although they were originated to explain the division of eggs with centrally located nuclei. Let us repeat them: The asters are spiny gel spheres and cleavage is the consequence of their separation by the elongation of the spindle.

There is, however, a contradiction. For if an excentric spindle simply elongates in the ordinary fashion, even though the furrow forms earlier on the side where the rays are shorter, by the time cleavage is nearly completed the blastomeres ought to assume the shape shown in Figure 4. Since this never happens in actual cleavage, some other factor must be operating.
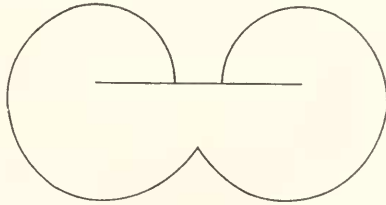

FIGURE 4.   The probable shape of the blastomeres if an excentric spindle should simply elongate without bending.

Perhaps another model, this time one with the spindle and astral centers located above the middle of the egg, will help to explain this factor (Fig. 5, *A*). If the asters are pushed apart and their positions fixed by putting a pin through the holes in the bars representing the spindle, these automatically form an angle (Fig. 5, *B*). Since no special rotating force is being applied under this circumstance, the flexion of the "spindle" must be the direct result of the simple separation of the "asters." If this model experiment conveys a correct picture, it leads directly to the suggestion that the spindle may be bending during the cleavage of *Astriclypeus* eggs.

In the model, the mechanical force causing the flexion of the bars is very easy to understand. The tips of the rays (bamboo rods) are attached to the cortex (rubber band). Therefore, when the astral centers are pushed away from each

other by a given distance, the tips of the rays are left behind by that distance in relation to the astral centers.   Under such a circumstance, the shorter rays have to rotate through larger angles than the longer rays in order to cover the shifted distance.   This difference between the rotating angles of the rays of the two sides will work, in the net result, so as to rotate the whole aster in the direction of the shorter rays, provided that the aster is rigid and can maintain a constant shape. In the model, these rotations naturally force the two bars, each attaching to one "aster," to make an angle between them.
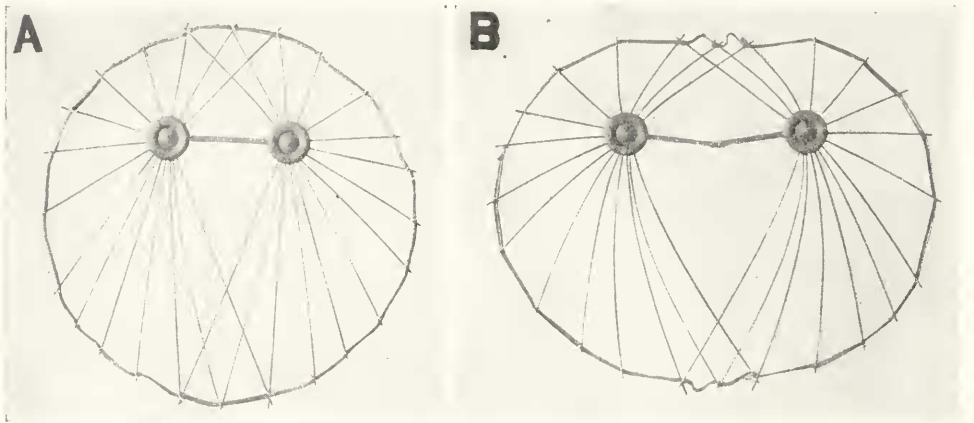

FIGURE 5.   A model similar to that shown in Figure 3, except that the spindle and the astral centers are made excentric (A).   In B, after separating the astral centers and fixing their positions by putting a pin through the holes in the bars, note the following points: (1) The fountain figure of the polar rays is obscured and the majority of the rays turn in the same direction in a vortex configuration (compare with Fig. 3B).   (2) The innermost pair of the crossing rays at the upper pole is practically straight while that of the lower pole is clearly bending and finally (3) the spindle is flexed in the middle.

The model, however, still cannot imitate the expansion phase, because, being two-dimensional, it cannot take care of the factor of the constancy of the cell volume. But, *a priori,* since the furrow surface is sucked into the gap between the two asters in the expansion stage, the rotation effect will be more emphasized: viz., during the expansion phase of actual cell cleavage, the spindle will bend more acutely, to such an extent that it will even tend to tear open the vegetal region, forming the furrow. It must be pointed out here that in order to allow the free rotation of the two asters around their excentric centers, the volume of the cells would have to be increased, as can be judged from Figure 6.   If the volume of the cell were kept constant, the rotation of absolutely rigid asters would be impossible.   The fountain-figured bending of the polar rays is also quite significant in this connection, as it makes possible astral rotation within cytoplasm of a constant volume.

SPINDLE BENDING IN ASTRICLYPEUS EGGS

The next step was to determine whether the spindle is actually bending in the *Astriclypeus* eggs.   Even a hasty examination was enough to confirm this fact.

On further investigation, it came to the authors' notice that though the degree of excentricity of the mitotic figure is fairly constant within a single batch of eggs, it varies somewhat among different batches, and the acuteness of the spindle bending is proportional to the degree of its excentricity. In speaking about the degree of excentricity, in order that the statement be accurate, the eggs must be observed strictly from the side.[1] Naturally, the cases with extremely excentric spindles are most convenient for analysis. In Figure 7 are given tracings of micro-photographs showing the mode of cleavage and the conditions of ray bending. Purposely, in the first and the last drawings of Figure 7, eggs with less excentric spindles are shown, while the second to the sixth are successive stages in the cleavage of a single egg with an extremely excentric spindle. In diagrammatization, however, the results of many other observations were taken into consideration.
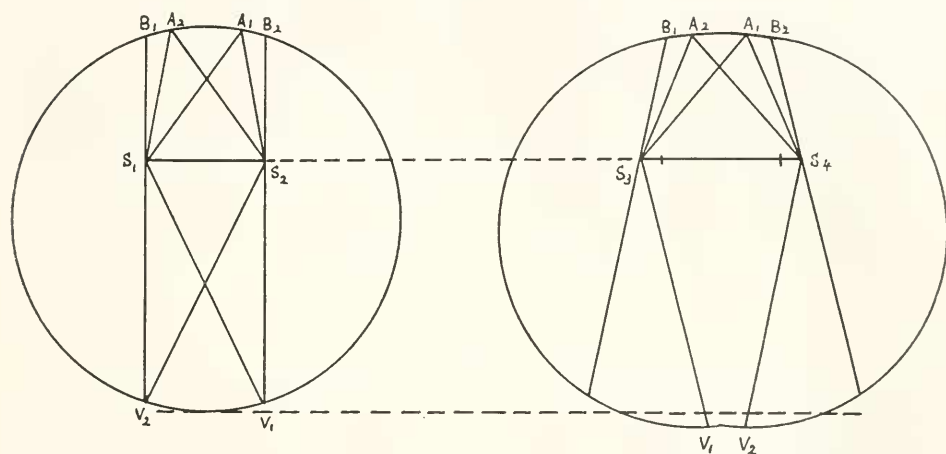

FIGURE 6. The hypothetical result of the simple rotation of asters around their excentric centers, as the result of the shrinkage of the upper pole. Notice the opening of the lower side accompanied by the stretching of the surface there and the bulging of the lower contour. In the actual cell division, such a perfectly free rotation is prevented because the cell volume is kept constant. But the rotation is allowed within a limit corresponding to the degree of bending of the polar rays.

In the first drawing, as far as could be judged from observation on living material and photographs, the astral rays and the spindle are both straight. (Remember that this is a case with less excentric spindle.) Theoretically, in this stage, the rotation of the aster must have begun, but this could not be ascertained, presumably because of its slightness. In the second drawing (an extreme case), the animal furrow is decidedly in the expansion phase. At this stage, two fan-shaped areas appear, one on each side of the cleavage furrow, in which the rays seem to be fewer than in other parts so that these regions are slightly more transparent and

[1] In order to obtain a strict side view of an egg, one takes advantage of the fact that the spindle excentricity is exceedingly constant within one batch. On surveying a culture of eggs with a medium magnification, one can easily get the idea of the maximum excentricity of the spindle as far as that particular batch is concerned. Such maximal cases must represent a full side view of the egg.
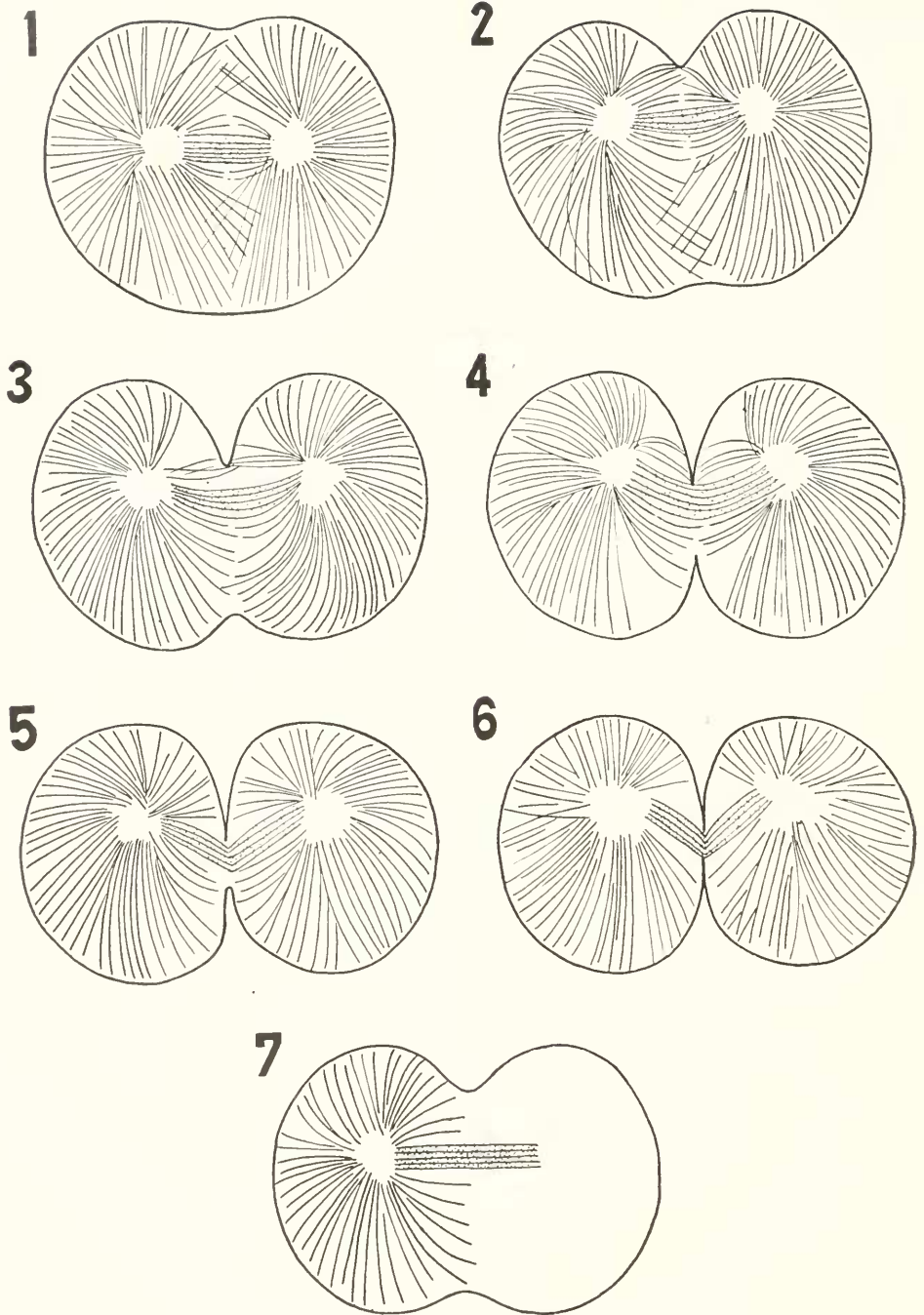
FIGURE 7. Tracings of micro-photographs of dividing *Astriclypeus* eggs showing the changes of curvature of the astral rays in successive stages. The first drawing is made from an egg of a different source but the second to the sixth drawings belong to a series of photographs of a

relatively bright.   Since the authors were at first skeptical about this fact, special attention was paid to this point, but numerous observations only served to confirm it.   According to the authors' interpretation, the appearance of the fan-shaped area is probably due to the fact that as the furrow begins to be drawn in by the suction effect, the formerly crossing rays are caught by the furrow constriction.   Then in the fan-shaped portion just behind the furrow tip, the rays will become relatively sparse.   This bundling of the equatorial rays by the furrow must certainly be the beginning of the formation of the so-called sheath rays, described by Yatsu in *Cerebratulus* eggs: "One striking feature of this stage is the formation of the sheath rays around the spindle, due to the fusion of the equatorial rays and a part of the intermediate rays.   The spindle shaped sheath rays seem to occur in a good many forms" (Yatsu, 1909, p. 387).   Simultaneously with the appearance of the fan-shaped area, the bending of the spindle becomes noticeable.

At the same time, two other changes are taking place in the curvature of the astral rays.   One is the bending of the vegetal crossing rays.   Among regularly dividing cells, the rays are straight while they are crossing, for they are supposed to be exerting traction force.   Therefore, the bending of these vegetal crossing rays deserves especial attention (see Fig. 5).   The other change is the curvature of the rays lying in the sector between the fan-shaped area and the spindle polar region. These rays, which have been either straight or bending in a fountain figure (see the right blastomere of the second drawing), now start to bend in unison into the same direction as the vegetal crossing rays, so that the rays of the whole aster display a beautiful vortical configuration (see the left blastomere of the second drawing). These changes in the way of bending of the rays can best be explained by thinking that the asters have been twisted around their centers.   In the third drawing, the sheath ray formation on the animal side, the spindle bending and the vortex figure all advance in degree and the vegetal crossing rays have almost been drawn apart. This drawing is of particular interest in two senses.   One is that it shows the vegetal half of the egg actually being torn apart by the twist.   The other is that it almost coincides with a drawing of Conklin's of the egg of *Cynthia partita* (Conklin, 1905; Pl. VII, Fig. 100).   In the fourth stage, the furrow on the animal side has almost reached the spindle and the sheath rays there begin to show a tendency to return to their original positions.   On the vegetal side, two fan-shaped areas can be recognized, indicating that sheath ray formation has begun there also.   Moreover, the formerly

single egg.   The spindle of this egg is more excentric than that of the first drawing.   The seventh drawing is an example of an egg with a far less excentric spindle.   Drawing 1, rays are practically straight.   Theoretically the asters must have begun to rotate, but this cannot definitely be ascertained.   Drawing 2, note the appearance of the fan-shaped clear areas on both sides of the animal furrow in the expansion phase; sheath ray formation; the bending of the vegetal crossing rays; the slight sign of spindle bending.   Drawing 3, further development of the fan-shaped bright portion; advance in the spindle bending and dissociation of the crossing rays of the vegetal side.   Note also the vortex configuration of the rays.   Drawing 4, approximate contact of the animal furrow with the spindle and the tendency of the rays of the animal side to return to a straight condition.   Appearance of the fan-shaped areas on the vegetal side. Drawing 5, last moment of division.   Disappearance of the fan-shaped areas and definite bending of the spindle.   Drawing 6, interkinetic stage.   Bending of the vegetal rays in the opposite direction (divergent bending).   Drawing 7, division of a cell with less excentric spindle. Notice usual fountain figure of the rays instead of the vortex configuration and apparent straightness of the spindle.

bent vegetal rays seem to have returned to a straight condition. But judging from the fact that the rays on the animal side still retain the same curvature as in the previous stage, this straightening of the vegetal rays may have been induced by the change in the shape of the aster itself.

In the fifth drawing, the cleavage is almost complete and all the rays, including the former sheath rays, are returning to a straight condition. At this stage, the spindle forms an obtuse angle with its apex directed toward the vegetal pole. The sixth drawing shows the egg in the interkinetic stage. Now the vegetal rays are turning in the opposite direction and new fan-shaped, ray-free areas appear adjacent to the now bent spindle. In Yatsu's last drawing, this kind of bending is seen in the rays of the animal side. This post-cleavage bending is what the senior author called "divergent bending" in the previous paper (Dan, K., 1943b), and it is considered to be an indication of the formation of the new surface (see also Dan and Dan, 1940).

In the seventh drawing, the condition within an egg whose spindle is not so excentric is illustrated. Here no vortex configuration is attained and the seemingly straight spindle and the usual fountain figure of the polar rays are seen as in *Cerebratulus* eggs. In this latter form, the spindle is not so excentric and its bending, consequently, becomes apparent only in much later stages, as can be judged by Yatsu's figure.

These observations on the eggs of *Astriclypeus* with extremely excentric spindles help to elucidate the intrinsic mechanism of spindle bending. It seems almost certain that the causative force of spindle bending is derived from the advancing animal furrow and transmitted by the sheath rays to the astral centers which, in turn, impart it to the spindle. But since the advance of the furrow is, itself, nothing but the result of the pushing apart of the two asters by the elongating spindle, an interesting cycle is seen. Thus it can be said that the causative force of spindle bending comes from the spindle's own elongation.

It must be emphasized that the fundamental situation is not at all different from that of typical cleavage. In both cases, sheath ray formation occurs. But in typical cleavage, since the inward pressure which produces sheath ray formation occurs at both the animal and vegetal sides simultaneously, the two effects cancel each other. In the heart-shaped cleavage, the pressure begins at the animal side and for a short time there is nothing to counteract it on the vegetal side. During this time the elongating spindle will encounter resistance on one side only, and will become concave toward that side.

Two other important facts are patent in these observations. One is that the connection between the aster and the spindle is an unexpectedly firm one. There is a striking correlation between the degree of astral rotation and the degree of spindle bending. If this junction were loose, the aster might rotate more, but the spindle could not be bent at all. The second fact is that the spindle must have a certain degree of rigidity, since it can be bent by applying forces at its two ends. The present data give further, and more decisive, support to the possibility postulated by K. Dan (1943a).

Lastly, it must be pointed out that although the model experiment helped to reveal the mechanism of spindle bending, the spindle bending during the shrinkage phase shown by the model could not be demonstrated within the living cell. In actual observation, the sheath ray formation is always seen as the direct impetus for

spindle bending.   Then is it permissible to apply this idea, obtained from a model, to actual cells?

The Effect of Astral Rotation on Surface Behavior

In the foregoing sections, it was argued from the model experiment that the cause of astral rotation lies in the difference in length of the astral rays on the two sides of an aster.   If this is so, the rotation of the aster must be occurring from the very beginning of the cleavage process.   Although the observations of the bending of the rays and of the spindle, such as were reported in the previous section, are quite decisive, they can be applied with certainty only to the expansion phase in which the sheath ray formation becomes evident.

For information on the early part of the cleavage process it was thought that a study of surface behavior might be used to establish some unmistakable evidence of astral rotation.   Here the kaolin method and its geometrical analysis were employed again.   The purpose of the analysis was to test whether or not the furrow formation is really due to the traction effect of the crossing rays.   The procedure was to make a sketch of an egg in the spherical condition on which the positions of the kaolin particles and of the astral centers were recorded.   From this sketch, the lengths of the crossing rays could be obtained.   Once these lengths were fixed, given the spindle length of each stage, the theoretical positions of the particles could be obtained for later cleavage stages.   These theoretical positions of the particles could then be compared with their observed positions.   If the authors' postulates be correct two conditions follow: (1) The theoretical positions of the particles ought to fall on the sketched contour of the cleavage furrow because the surface is being pulled in by the rays during the shrinkage phase and (2) the degree of shrinkage in the distance between the two theoretical points ought to agree with that of the observed particle-distance as long as the particle-distance coincides with the real crossing range of the rays.   In the previous paper of this series, it was shown that the above propositions satisfactorily hold in actual cases (Dan, K., 1943b; Figs. 6 and 7, Table III).   But it must be stressed that even when the second condition fails, the first condition proves to be true as long as the deviation between the actual particle-distance and the crossing range is not too great.   This means that the second proposition is a stricter test for the hypothesis than the first one.   However, in order to apply the second proposition properly, the painstaking accumulation of many readings is necessary to map out beforehand the actual range of crossing of the rays (see Dan, K., 1943b; Fig. 8).   On the contrary, the first proposition, although it is less sensitive, is convenient and quite satisfactory as a qualitative test. When the suction force develops and the crossing rays are pulled apart, the intersection points (apices of the triangles) begin to fall outside the contour of the cleavage furrow.   The time at which the intersection points begin to deviate is always simultaneous with the onset of the expansion phase.

Now let us see how the surface of *Astriclypeus* eggs behaves in the kaolin experiment.   The method has been used on these eggs previously (Dan, Dan and Yanagita, 1938) but the present observations are especially concerned with eggs containing extremely excentric nuclei.   Two sets of the records for the qualitative test are given in Figures 8 and 9 and Table I.   As for the animal furrow, in the egg shown in Figure 8, the expansion phase sets in at the fifth stage and at this very
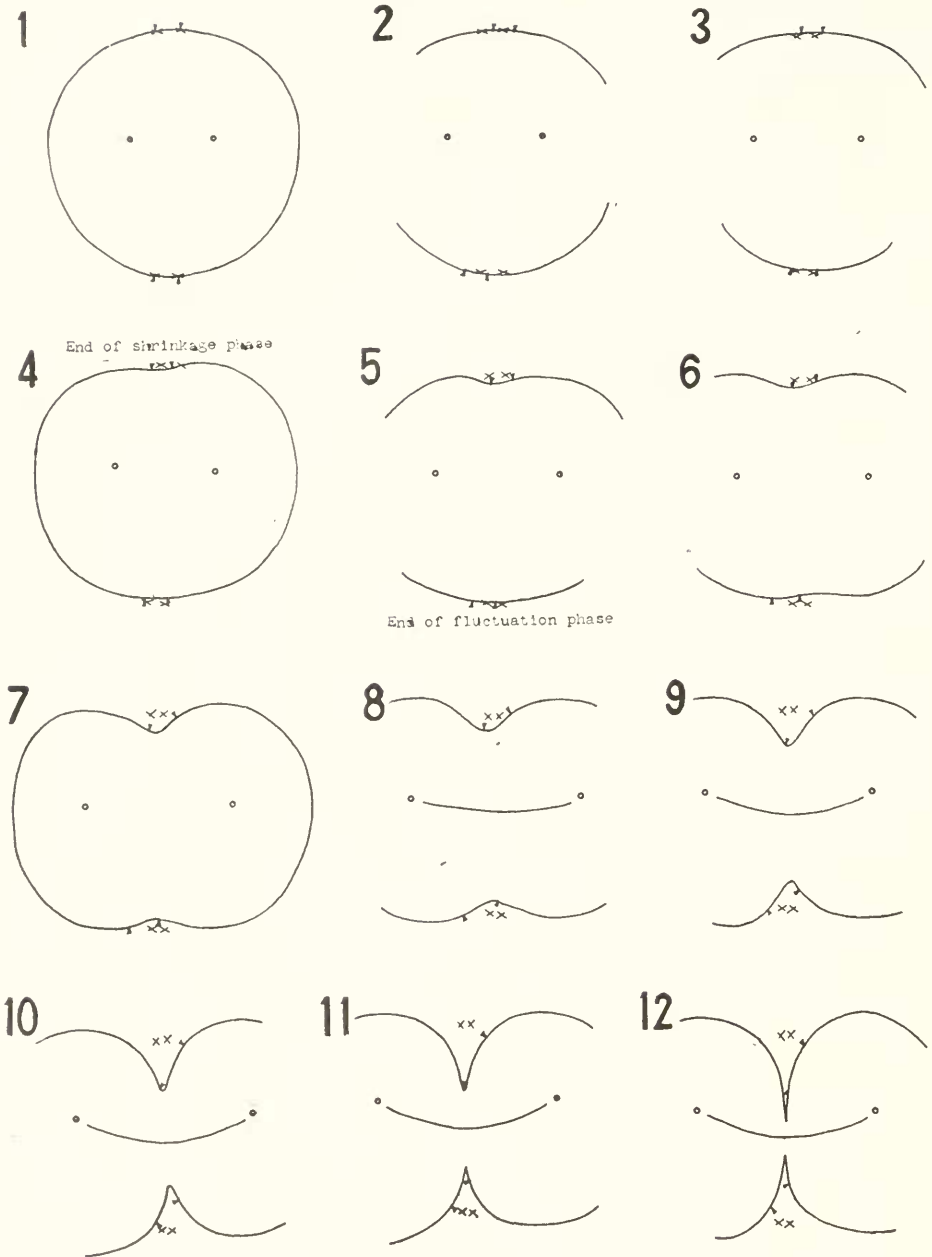
FIGURE 8. Successive camera lucida drawings of a cleaving *Astriclypeus* egg with the recorded positions of kaolin particles (▼▼) and astral centers (○○). In the spherical condition (first drawing), the two particles at the animal pole and at the vegetal pole respectively are connected to the astral centers by four hypothetical rays (which, however, are omitted from the figures). In succeeding drawings, by using the sets of four lines obtained in the first drawing as radii and taking the astral centers of successive stages as centers, two intersection points

stage, the intersection points start to deviate from the contour. In the egg shown
in Figure 9, this happens at the sixth stage and the coincidence between the onset
of the expansion phase and the initiation of the deviation of the intersection points
from the contour is again unfailing. As far as the vegetal furrow is concerned, in
the egg of Figure 8, the particle-distance follows a zig-zag course: 100.0%–97.9%–
111.4%–96.9%–104.1%. At the sixth stage, the value jumps to 121.8% which is
followed by a further rise. Therefore, the sixth stage can be considered as the
beginning of the expansion phase. In the egg of Figure 9, although the course of
change is more gradual, the same tendency is unmistakably recognizable: 100.0%–
104.0%–103.0%–104.3%–104.6%–100.0%–100.3%. At the eighth stage, the value
goes up to 115.2% and the intersection points begin to fall outside the furrow con-
tour. These examples indicate that in eggs with extremely excentric spindles, the
initial shrinkage effect is so obscured at the vegetal side that the term "shrinkage
phase" is no longer appropriate. For this reason, in the present paper, the term
"fluctuation phase" will tentatively be used. But a rather surprising fact is that
in Figure 8 the intersection points coincide well with the traced cell contour during
this fluctuation phase. In Figure 9, the intersection points do not at least go out-
side the drawings until the end of the fluctuation phase. From these facts, it
seems permissible to conclude that at both poles of the cleaving *Astriclypeus* eggs,
fundamentally the same division mechanism is at work.

Then why is this expressed as shrinkage at the animal side and alternate shrink-
age and expansion at the vegetal side? It may be due to the rotation of the asters.
It was suggested in the foregoing section that when the two asters rotate so that
their animal hemispheres converge, the vegetal furrow must tend to be torn open.
In reality, the situation may be somewhat more complicated. While the asters turn,
if the tips of the crossing rays can retain their attachment to the cortex, the vegetal
surface may be made to shrink more than when the rotation is absent. But if these
rays pull loose from the cortex and the asters become free to rotate, they may stretch
the surface between them on rotation as is diagrammatically shown in Figure 6.
If the rays pull loose in several steps shrinkage and expansion will alternate.

Another point which is rather peculiar is the shift of the intersection points to
the inside of the cells in the drawings, a form of behavior which has never been met
with in regularly dividing cells. For example, in Figure 9, during the fluctuation
phase of the vegetal furrow which lasts for seven stages, the intersection points fall
inside the cells at two different times including four stages. Stage 3 and stage 5 of
Figure 9 are of special interest. In those two stages, the animal furrow being still
in the shrinkage phase, the intersection points of that side are coinciding with the
contour. As long as the intersection points of the animal side are coinciding with
the contour, one is provided with a guarantee that no error is involved in the re-
cording of the positions of the astral centers. Yet the vegetal intersection points
fall inside the drawing. This shows that the inward shifting of the vegetal inter-
section points is not an error but represents something which is actually happening

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(××) are found for each pole. The percentage changes of the observed particle-distance (P)
and of the theoretical distance between the two intersection points (D) are given in Table I to-
gether with the time of recording. Note that during the shrinkage phase of the animal pole
(from the 1st to the 4th stage) and the fluctuation phase of the vegetal pole (from the 1st
to the 5th stage), the intersection points fall on the egg contour.

in *Astriclypeus* eggs. There are two suggestions as to its identity. One is spindle bending. Since the bent spindle is convex toward the vegetal pole, it may push out the contour of the vegetal furrow. The other is that when the asters rotate around their excentric centers, pulling loose their crossing rays, the vegetal contour may bulge out as is indicated in Figure 6. At any rate, the astral rotation may
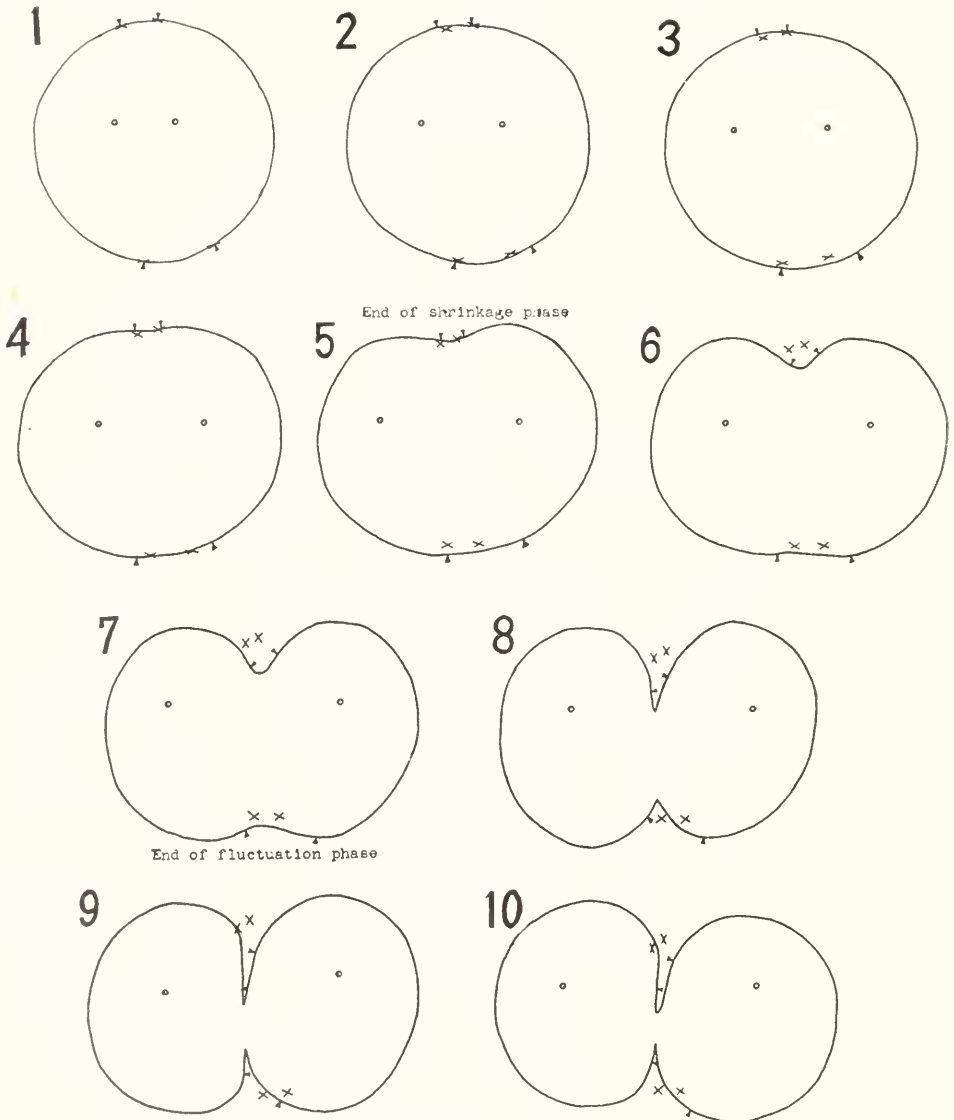

FIGURE 9. Another example of the geometrical analysis of an egg with extremely excentric spindle. The shrinkage stage of the animal pole lasts for five stages and the fluctuation of the vegetal pole ends at the seventh stage. Note that the vegetal intersection points are sometimes inside the contours.

TABLE I

The results of the geometrical analyses of *Astriclypeus* eggs with extremely excentric spindle shown in Figures 8 and 9, exemplifying the "fluctuation phase." P, percentage changes of observed particle-distance. D, percentage changes of theoretical particle-distance.

The case of Figure 8

Stage	1	2	3	4	5	6	7	8	9	10	11	12
Time of recording at 21.0° C.	0″	1′00″	2′00″	3′00″	3′50″	4′40″	5′00″	5′45″	7′40″	8′38″	9′30″	10′35″
Animal furrow	P=100.0 D=100.0	P=93.7 D=88.5	P=92.7 D=75.0	P=83.3 D=83.3	P=97.9 D=65.6	P=119.7 D=61.4	—	—	—	—	—	—
Vegetal furrow	P=100.0 D=100.0	P=97.9 D=91.6	P=111.4 D=90.6	P=96.9 D=85.4	P=104.1(?) D=69.7	P=121.8 D=63.5	P=127.0 D=61.4	—	—	—	—	—

The case of Figure 9

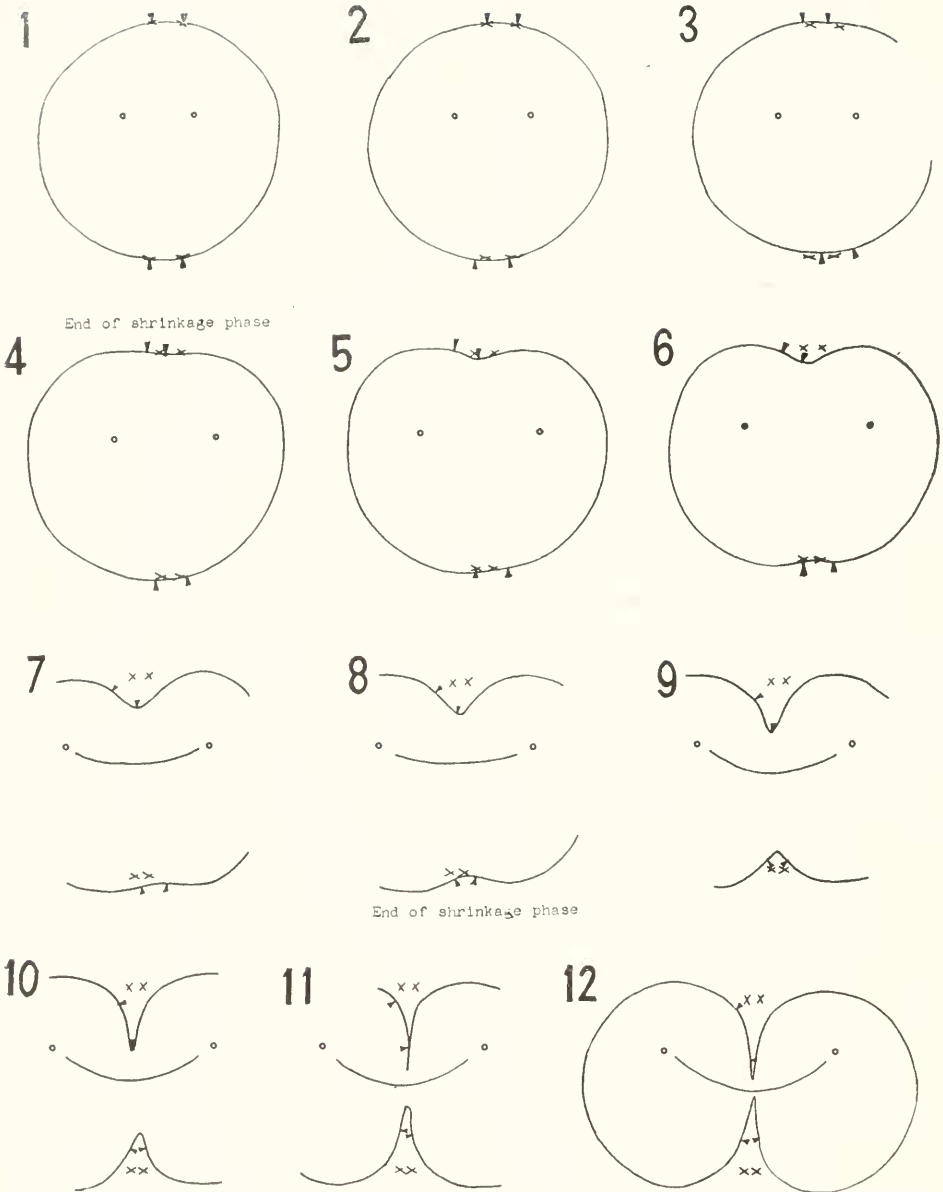| Stage	1	2	3	4	5	6	7	8	9	10
Time of recording at 26.5° C.	0″	2′00″	2′50″	4′05″	5′00″	7′00″	8′05″	9′30″	10′45″	12′30″
Animal furrow	P=100.0 D=100.0	P=94.0 D=73.5	P=80.8 D=68.8	P=72.8 D=59.6	P=66.9 D=47.0	P=99.3 D=43.0	P=114.5 D=39.1	P=158.9 D=39.7	P=185.4 D=39.7	P=218.5 D=39.7
Vegetal furrow	P=100.0 D=100.0	P=104.0 D=73.3	P=103.0 D=63.0	P=104.3 D=55.4	P=104.6 D=45.5	P=100.0 D=39.3	P=100.3(?) D=33.0	P=115.2 D=31.7	P=122.1 D=33.0	P=123.8 D=30.4

FIGURE 10. Geometrical analysis of an egg with a less excentric spindle. Both the animal and the vegetal poles undergo a shrinkage. The vegetal intersection points are inside the drawings at the seventh and the eighth stages with bent spindles.

TABLE II

Data of the geometrical analysis of an *Astriclypeus* egg with a less excentric spindle (see Fig. 10). Designations same as Table I.

Stage	1	2	3	4	5	6	7	8	9	10	11	12
Time of recording at 22.5° C.	0″	1′00″	2′15″	2′30″	3′15″	4′00″	5′00″	6′40″	7′35″	8′35″	10′00″	11′35′
Animal furrow	$P=100.0$ $D=100.0$	$P=90.6$ $D=88.2$	$P=78.1$ $D=88.2$	$P=58.5$ $D=66.4$	$P=85.1$ $D=54.6$	$P=75.0$ $D=56.2$	$P=107.8$ $D=46.8$	— —	— —	— —	— —	— —
Vegetal furrow	$P=100.0$ $D=100.0$	$P=100.0$ $D=97.7$	$P=98.4$ $D=88.7$	$P=93.2$ $D=73.1$	$P=97.7$ $D=62.4$	$P=91.7$ $D=63.9$	$P=83.4$ $D=50.3$	$P=81.9$ $D=50.3$	$P=84.2$ $D=45.8$	$P=121.0$ $D=45.1$	— —	— —

offer the best single explanation for both the fluctuation phase and the inward shift of the vegetal intersection points.

In the second paper of this series, the authors (Dan, Dan and Yanagita, 1938) handled the data of the kaolin experiments on the eggs of this sand-dollar as if there was no qualitative difference between the behaviors of the two poles of the cell. Even though they are sure that this omission did not affect the conclusion of that paper materially since they were not limiting themselves to the extremely excentric cases, they wish to make this point clear.

Finally, the eggs with less excentric spindles must be examined. In one sense, this test is very important because in eggs with less excentric spindles, their bending can be noticed only in a very late stage of the cleavage process. The data are given in Figure 10 and Table II. As can be seen from the table, the particle-distance on the animal furrow reaches its minimum at the fourth stage. Until this time, the intersection points remain on the contour. The vegetal furrow, in this case, undergoes a shrinkage similar to other examples reported in the previous paper. In this particular case, the vegetal furrow remained in the shrinkage phase for an exceptionally long time, i.e., until the eighth stage. Yet the intersection points stay on the contour through this period, except the seventh and the eighth stages where the vegetal intersection points are inside the contours concurrent with the onset of spindle bending. On closer examination of Table II, however, it will be noticed that the degree of shrinkage is much less for the vegetal furrow (minimum—81.9%) than for the animal furrow (minimum—58.5%). Is this, then, due to the pulling loose of vegetal rays as a result of astral rotation? Here, the stricter test—i.e., the numerical fit between the theoretical and observed degrees of shrinkage—must be resorted to. It was pointed out that these figures coincide only when the positions of the kaolin particles fall exactly at the tips of the crossing rays. This occurs purely by chance. But if numerous records are examined, such cases will inevitably be met with. This is true with the animal pole of the *Astriclypeus* eggs as shown in Table III and Figure 11.

On the contrary, for the vegetal furrow, no case of an exact fit has been found in the entire mass of records. In every case, the degree of the observed shrinkage was far less than that of the theoretical shrinkage, no matter how small a particle-distance was chosen. (When a particle-distance is wider than the actual crossing range, the observed degree of shrinkage becomes less than that of the theoretical shrinkage (consult Figure 2).) The case given in Figure 10 and Table II well exemplifies the situation. This is decisive enough evidence that the asters must

TABLE III

An example of a nearly perfect fit between the observed (P) and the theoretical (D) degrees of shrinkage at the animal furrow of the *Astriclypeus* egg (see Fig. 11).

Stage	1	2	3	4	5	6
Time of recording	0''	2'05''	3'10''	4'00''	6'25''	6'55''
Observed shrinkage	$P = 100.0$	$P = 93.0$	$P = 89.5$	$P = 83.7$	$P = 73.2$	$P = 131.3$
Theoretical shrinkage	$D = 100.0$	$D = 96.5$	$D = 88.3$	$D = 81.3$	$D = 69.8$	$D = 58.1$

be rotating also in the eggs with less excentric spindles where the early detection of spindle bending is difficult.

In short, the above result indicates that in the early stages of cleavage of the cases of less excentric spindles, the asters are rotating although spindle bending is not detectable.   This may mean that while, as was pointed out above, the connection between the spindle tip and the astral center is surprisingly firm, it is not absolutely rigid and a certain degree of astral rotation may be induced by the pressure of the furrow on the sheath rays before the spindle is noticeably affected.

Spindle Bending in Other Forms

The bending of the spindle in *Astriclypeus* has been discussed and is shown diagrammatically in Figure 7.   Other observations of spindle bending are not scanty in the literature.   Yatsu's last drawing clearly illustrates it for *Cerebratulus*. Among other egg cells, Conklin (1902) shows many drawings of spindle bending in
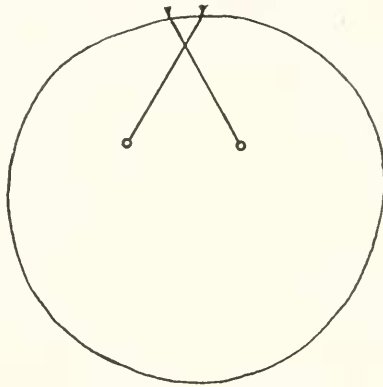

FIGURE 11.   Sketch of the egg, the data of which are given in Table III, showing the range of crossing of the rays at the animal pole.

Crepidula eggs (Plate III, Figs. 60, 61; Plate V, Figs. 80, 81), Kuhn (1913) reported it in the summer eggs of *Polyphemus* (Fig. 15) and Rhumbler (1901) in the eggs of *Rhabdonema* (?).   From both the drawings and the original texts of these reports, it is evident that the spindles lie excentrically in all cases.

Examples of spindle bending are also found among male sex cells.   Wilson takes up this subject in his book (1925, p. 140) in connection with the division of the spermatogonia of the grasshopper, *Rhomaleum*.   Among recent workers, Sato (1940) touches this point in the spermatogonia (Figs. 18, 48) and the spermatocytes (Figs. 20, 50) of the scorpion, *Buthus martensii*, and he discusses it as a possible cause of the rosette arrangement of these cells within their cysts.   He kindly informed the authors in a personal communication that, in these cases, the position of the resting nucleus and subsequently of the spindle is excentric.   He also asserted that the bending of the spindle is not due to a lack of space within the cyst.   From the above references, the authors believe there is enough evidence to support the proposition that spindles bend whenever they occur excentrically in cell bodies.   As a matter of fact, once aware of this situation, careful observation can frequently

catch slight bending of the spindle even in the so-called "regularly dividing" sea-urchin eggs, in batches in which the spindle lies more or less off the geometrical center of the cell (*see* Fry, 1937; Figs. 1–5). Another fact of special importance is that some of the investigations cited above include figures of spindle bending which occur well before a cleavage furrow is formed, thus definitely eliminating the possibility that the spindle is passively bent inward by the advancing cleavage furrow.

Artificial Induction of Spindle Bending

The foregoing analysis furnishes evidence that the cause of spindle bending is an unbalanced condition of the mechanical strains between the animal and vegetal sides of the spindle. If such is the case, by experimentally causing a similar unbalanced condition of force, it should be possible to induce spindle bending in a material in which it is not seen normally. This is achieved by the use of ether. The eggs of any kind of echinoid which normally divide in a symmetrical fashion can be used. The eggs are fertilized and a short time before the first cleavage is due, the surrounding sea-water is changed to an ether-sea-water mixture (0.6% by volume).
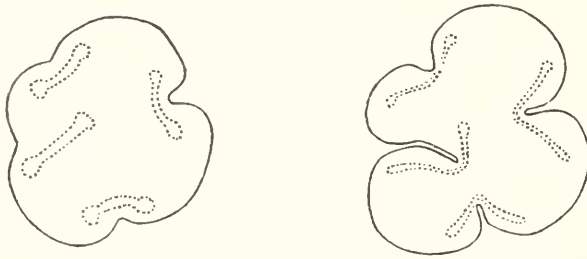

Figure 13. Reproduction of Yatsu's Figures 22 and 23 of a *Cerebratulus* egg, whose previous mitoses were inhibited by compression, about to cleave into four binucleate cells. The bending of the spindle is clearly shown.

In ether, even though the nuclear division takes place, the cytoplasmic division is suppressed and binucleated cells are obtained (Wilson, 1901). If these eggs are later washed free from ether, at the second cleavage, two spindles are formed side by side. Under this circumstance, the cleavage furrow appears in a plane at right angles to these spindles and two binucleate cells result. As the furrow constricts the cell, each of the two parallel spindles will obviously be cut by the furrow only on its peripheral side. In the living condition, during the cleavage, two obtusely V-shaped spindles can be seen which, at the final stage, are held in contact at their apices, giving the form of an X. Sections of ether-treated *Strongylocentrotus* eggs at the second division are shown in Figure 12.

In the literature, Boveri (1910) sketched a bent spindle in an *Ascaris* egg, the cleavage furrow of which was suppressed on one side by centrifugation. Yatsu (1908), in his experiments on *Cerebratulus* eggs with a compressorium, shows clear figures of spindle bending in an egg which is dividing simultaneously into four binucleate cells, even though his interest in that paper is on other points. Two of his figures are shown in Figure 13. This example suggests that whenever a cell divides simultaneously into many cells, spindle bending must be involved.

Discussion

In the foregoing sections, the authors have tried to analyse the cause of the bending of excentric spindles, and they have attributed it to the unbalanced mechanical resistance on the two sides of an elongating spindle resulting from its ex-
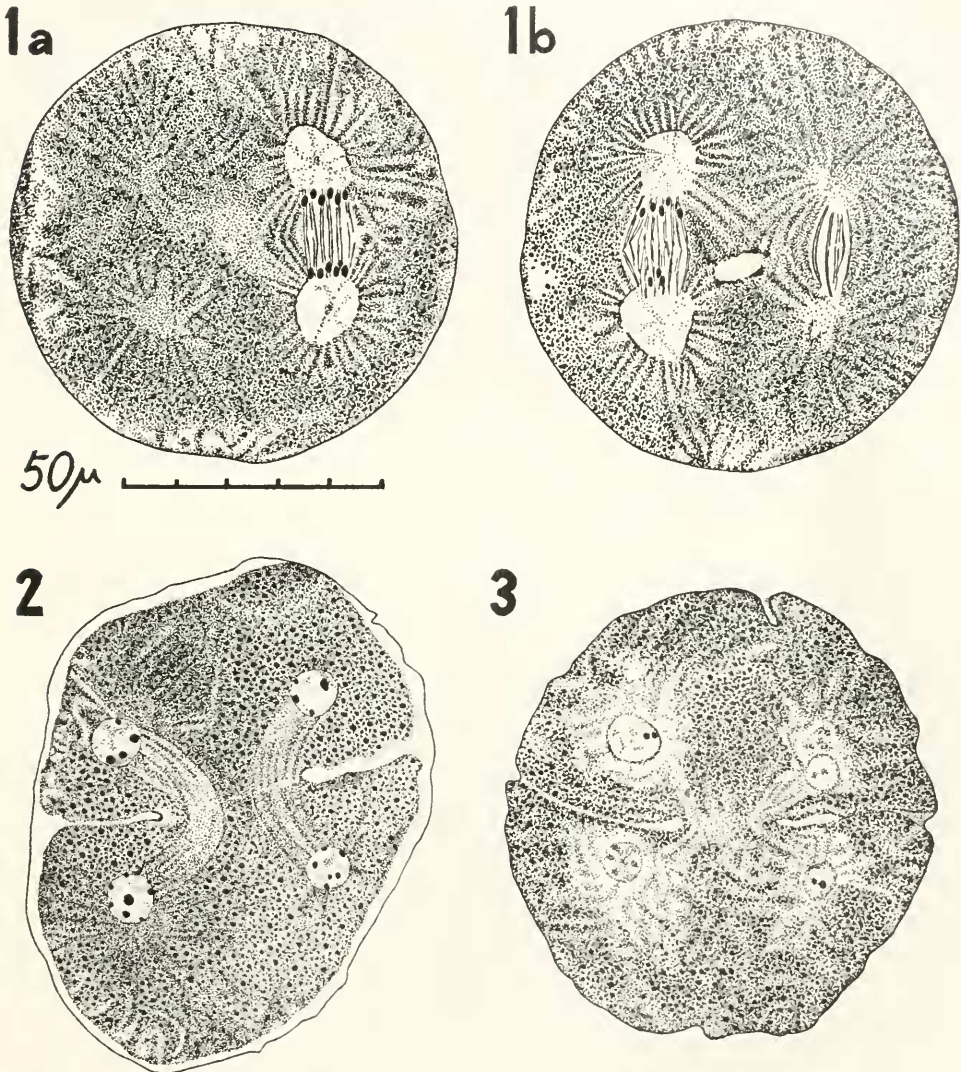

FIGURE 12. Sections of an egg of *Strongylocentrotus pulcherrimus* in the second division, the first cleavage of which was suppressed by ether. Fixed in chrome-formol mixture, 1a and 1b are two successive sections of an egg showing two spindles and four asters (the slit in the middle of 1b is an artefact). Two is an egg cleaving into two binucleate cells with the bending of the spindles. Three is another egg in a slightly later stage with sharply bent spindles, the spindles being much more vague in this case.

centricity.   As far as the authors are aware, Gray is the only investigator who has
tried to derive an explanation of this mode of division from a general theory on the
division mechanism of regularly dividing cells.   In discussing "astral cleavage"
(Gray, 1931, pp. 201–202), he writes: "If the line joining the center of the two
asters does not pass through a diameter of the egg by the time the astral rays reach
one side of the egg, then the cleavage furrow develops first on that side, and only
later (as the rays reach the opposite side of the equator) does the furrow develop on
the other side. . . . That the irregularity of form and position of the asters is the
cause and not the result of irregular cleavage is suggested by the fact that similarly
irregular cleavages can be induced to occur in *Echinus* eggs by experimental means."

Although the authors readily support the latter part of his statement, they can-
not help thinking that the mechanism he offers as the explanation for the formation
of one-sided furrows is quite unsatisfactory.   In the first place, his theory requires
the existence of a stage in which the rays are reaching the nearer periphery but not
the more distant side.   This is quite contrary to the observed facts.   It is obvious
that the furrow formation starts with the elongation of the cell body only after the
astral rays have filled up the cell interior.   In the second place, even allowing the
first requirement of Gray's theory, the way in which the furrow appeared on the
nearer side by his mechanism would not be the formation of a furrow in the real
sense of the words but would rather be the bulging out of the two sides of the
furrow.   Consequently, at the completion of such cleavage, the egg should have
made headway toward the animal side, causing the spindle to take a central position.
This is utterly incompatible with the observed facts.

A striking similarity between *Astriclypeus* and *Cynthia* eggs concerning spindle
bending and the vortex configuration of the astral rays has been mentioned.   How-
ever, the real situation seems to be much more complicated.   Conklin (1905) states
that the cleavage furrow of the 1st division starts from the vegetal pole near which
the clear protoplasm and the mitotic apparatus are lying excentrically (*see* Conklin,
1905; Pl. II, Fig. 20).   So far nothing contradicts the authors' expectations.   But
he further says that when the spindle begins to bend, toward the end of this cleavage,
it becomes convex toward the vegetal pole from whence the furrow first appeared
(Pl. VII, Fig. 100).   He moreover asserts that this is also true for the first cleav-
age of *Ciona intestinalis* (Pl. XI, Fig. 198).   The direction of this bending is quite
opposite to the one which can be expected from our theory.

On the other hand, for the second and third cleavages, the direction of the
spindle bending is exactly what we would anticipate.   He writes: "In the second
cleavage the constriction of the cell begins at the periphery or free surface and pro-
ceeds inward through the cell body (figs. 104, 105).   . . . and the middle of each
spindle is bent in toward the center of the egg (fig. 105)" (p. 44).   On page 46, he
has another sentence to the same effect in connection with the first cleavage and with
Figures 106, 107.   A reexamination of ascidian egg cleavage is urgently needed.

From what has been discussed concerning the cause of spindle bending, it
might be concluded that spindle bending is impossible in ordinary plant cells in
which neither astral rays nor cleavage furrows are found.   In spite of this, there
are cases in which spindle bending has been reported among plant cells.   Professors
Sinotô and Wada of the Botany Institute kindly informed the authors of two such
cases.   One is in the pollen mother cells of a haploid *Triticum* reported by Gaines
and Aase (1926) and the other is in the pollen mother cells of hybrid offspring of

Triticum and *Aegilops* studied by Kihara and Lilienfeld (1932). The cause of such bending may be quite different from that among animal cells and it must probably be sought for in the intrinsic make-up of the spindle itself.

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Summary

1. Study was made of the cleavage of cells with excentric mitotic figures.

2. In the division of such cells, (a) the cleavage furrow appears from the side of the cells nearest to the mitotic figure, (b) the asters rotate, causing the animal hemispheres to converge, and (c) the spindle bends, with the convex side directed toward the vegetal pole.

3. This situation can be explained by thinking that the mechanical resistance for the elongating spindle is different in magnitude on its two sides as a result of the excentricity of the mitotic figure.

4. The above situations can be artificially induced in materials which never show them normally, by producing a similar unbalanced resistance on the two sides of the spindle.

5. Gray's theory concerning the mechanism of the formation of one-sided cleavage furrows is discussed.

Literature Cited

Boveri, Th., 1910. Ueber die Teilung centrifugierter Eier von Ascaris megalocephala. *Arch. f. Entw.-mech.,* **30**: 101.

Conklin, E. G., 1902. Karyokinesis and cytokinesis in the maturation, fertilization and cleavage of Crepidula and other gasteropoda. *Jour. Acad. Nat. Sci. Philadelphia,* Sec. ser., **12**: 1–122.

Conklin, E. G., 1905. The organization and cell-lineage of the Ascidian egg. *Jour. Acad. Nat. Sci. Philadelphia,* 2nd ser., **13**: 1–126.

Dan, K., T. Yanagita, and M. Sugiyama, 1937. Behavior of the cell surface during cleavage I. *Protoplasma,* **28**: 66–81.

Dan, K., J. C. Dan, and T. Yanagita, 1938. Behavior of the cell surface during cleavage II. *Cytologia,* **8**: 521–531.

Dan, K., and J. C. Dan, 1940. Behavior of the cell surface during cleavage III. On the formation of new surface in the eggs of Strongylocentrotus pulcherrimus. *Biol. Bull.,* **78**: 486–501.

Dan, K., 1943a. Behavior of the cell surface during cleavage V. Perforation experiment. *Jour. Facult. Sci. Tokyo Imp. Univ.,* sec. IV, **6**: 297.

Dan, K., 1943b. Behavior of the cell surface during cleavage VI. On the mechanism of cell division. *Jour. Facult. Sci. Tokyo Imp. Univ.,* sec. IV, **6**: 323.

Fry, H. J., 1937. Studies of the mitotic figure. VI. Mid-bodies and their significance for the central body problem. *Biol. Bull.,* **73**: 565–590.

Gaines, E. F., and H. C. Aase, 1926. A haploid wheat plant. *Amer. Jour. Botany,* **13**: 373–385.

Gray, J., 1931. *Experimental cytology.* Cambridge University Press.

Kihara, H., 1932. Genomanalyse bei Triticum und Aegilops. Kihara, H. und F. Lilienfeld, 1932. IV Untersuchungen an Aegilops × Triticum- und Aegilops × Aegilops-Bastarden. *Cytologia,* **3**: 384–456.

Kühn, A., 1913. Die Sonderung der Keimbezirke in der Entwicklung der Sommereier von Polyphemus pediculus de Geer. *Zool. Jahrb. Anat. und Ontogen,* **35**: 243–340.

Rhumbler, L., 1901. Ueber ein eigentümliches periodisches Aufsteigen des Kernes an die Zelloberfläche innerhalb der Blastomeren gewisser nematoden. *Anat. Anz.,* **19**: 60–88.

Sato, I., 1940. Studies on the cytoplasmic phenomena in the spermatogeneses of the oriental scorpion, Buthus martensii, with special reference to the structure of the chondriosome ring and the dictyokinesis. *Jour. Sci. Hiroshima Univ.,* ser. B. div. 1, **8**: 1–116.

Wilson, E. B., 1901. Experimental studies in cytology II and III. *Arch. f. Entw.-mech.,* **13**: 353.

Wilson, E. B., 1925. *The cell in development and heredity.* New York.

Yatsu, N., 1908. Some experiments on cell-division in the egg of Cerebratulus lacteus. *Annot. Zool. Jap.,* **6**: 267–276.

Yatsu, N., 1909. Observations on ookinesis in Cerebratulus lacteus, Verrill. *Jour. Morph.,* **20**: 353–402.

Ziegler, H. E., 1898. Experimentelle Studien über die Zelltheilung. *Arch. f. Entw.-mech.,* **7**: 34.